[illegible] of [illegible] variance

Prepared versions

Version 1.0 ⟶ A only
Version 2.0 ⟶ B only
Version 3.0 ⟶ A, B no overlap
Version 4.0 no A, B

Version
oligos A8333C11
A8333C10
diluted to 100 pmol/μl
50 μl each mixed
↓ 94 ⟶ 40 slow cool

Ligation 7 μl oligos (100 pmol)
2 μl 4236 DNA
7 μl H_2O
1.5 μl 10X buffer
1.5 μl 10mM ATP
1.0 μl Ligase
15 μl
↓ 16° 1 hr.
↓ 65°C 15'

[redacted]

[illegible]
[illegible]

ProdiGene Promoters

Name	Description		Reporter	Test vector		Transform	Results
PGNpr1	maize polyubiquitin 1 (UBI1)		GUS	pPHP8904		Corn	GSA
			GUS-6xhis	pPGN7062		Corn	GSB
PGNpr2	maize globulin 1		GUS-6xhis	pPGN9025		Corn	
PGNpr3	maize 27 kD alphazein		GUS-6xhis	pPGN9021		Corn	PMD
PGNpr4	maize UBI1 no heat shock elements (HSE): UbiC		GUS-6xhis	pPGN7547		Corn	GSC
PGNpr5	maize UBI1 no 3' HSE: UbiD		GUS-6xhis	pPGN7565		Corn	GSD
PGNpr6	maize UBI1 no 5' HSE: UbiE		GUS-6xhis	pPGN7583		Corn	GSE
PGNpr7	maize UBI1 no HSE overlap: UbiF		GUS-6xhis	pPGN7600		Corn	GSF
PGNpr8	maize UBI1 replace HSE with 3x Pyl seed specific element: UbiG		GUS-6xhis	pPGN8926		Corn	GSG
PGNpr9	teosinte polyubiquitin 1		GUS-6xhis	pPGN8984		Corn	GSI
PGNpr10	teosinte polyubiquitin 1a		GUS-6xhis	pPGN8985		Corn	GSM
PGNpr11	sorghum polyubiquitin 1		GUS-6xhis	pPGN8986		Corn	GSN
PGNpr12	maize glutathione-S-transferase 1 (GST1)		GUS-6xhis	pPGN8987		Corn	GSP
PGNpr13	synthetic promoter RsynD with 35S enhancer 5' (tested with maize Adh-1 intr		GUS-6xhis	pPGN9006		Corn	GSR
PGNpr14	synthetic promoter Rsyn7 with 35S enhancer 5' (tested with maize Adh-1 intr		GUS-6xhis	pPGN9007		Corn	GSS
PGNpr15	maize HRGP		GUS-6xhis	pPGN9016		Corn	SCA
PGNpr16	maize P promoter (tested with maize Adh-1 intron)		GUS-6xhis	pPGN9035		Corn	SCD
PGNpr17	modified version of Agro mannopine synthase (superMAS)		GUS	pPHP10336		Pea	GSH
PGNpr18	bean phaseolin		GUS-10xasp	pPGN3275		Pea	GSJ
			GUS-6xhis	pPGN5690		Pea	GSK
PGNpr19	maize UBI1 no 5' HSE with a little extended seq at 5' end (beyond pr6)		GUS-6xhis	pPGN9042		Corn	GS1
PGNpr20	rice glutelin (1.3Kb of 5' sequence)		GUS-6xhis	pPGN9056		Corn	PMA
PGNpr21	rice glutelin 2.3Kb (2.3Kb of 5' sequence)		GUS-6xhis	pPGN9057		Corn	PMB
PGNpr22	rice globulin 26KDa		GUS-6xhis	pPGN9060		Corn	PMC
PGNpr23	maize globulin 2		GUS-6xhis	pPGN9076		Corn	

[redacted]

GIBCO BRL Custom Primers

Certificate of Analysis

Primer 1:

Primer Name: UBI HSP VER. 1A
Researcher:
Primer Number: A8333C10 (C10)
Primer Length: 66
Sequence (5' to 3'): PAG ACG GCA CGG CAT CTC TGT CGC TGC CTC CAC CGT TGG ACT TGC TCC GCT GTC GGC ATC CAG AAA T

Molecular Weight µg/µmole:	21299.2	µg per OD:	31.3
Millimolar Extinction Coefficient:	678.6	nmoles per OD:	1.4
Purity	Desalted	OD's	39.3
Tm (1 M Na+)	86	µg's*	1234
Tm (50 mM Na+)	76	nmoles	57
% GC	60	Coupling Eff.	99%
Notes:			

Primer 2:

Primer Name: UBI HSP VER.1B
Researcher:
Primer Number: A8333C11 (C11)
Primer Length: 67
Sequence (5' to 3'): PTT TCT GGA TGC CGA CAG CGG AGC AAG TCC AAC GGT GGA GGC AGC GAC AGA GAT GCC GTG CCG TCT GC

Molecular Weight µg/µmole:	21697.4	µg per OD:	29.8
Millimolar Extinction Coefficient:	732.9	nmoles per OD:	1.3
Purity	Desalted	OD's	10.7
Tm (1 M Na+)	97	µg's*	319
Tm (50 mM Na+)	78	nmoles	14
% GC	62	Coupling Eff.	98%
Notes:			

UBI 1A 57 nmoles 570 µl → 100 pmol/µl

14 nmoles 140 µl → 100 pmol/µl

*-See Note about Quantities in Supporting Information.

LIFE TECHNOLOGIES.

GIBCO BRL Custom Primers
Certificate of Analysis

Primer 1:

Primer Name: UBI HSPA VER.2A
Researcher:
Primer Number: D0373B07 (B07)
Primer Length: 61
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CCC TCT CGA CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	25105.2	µg per OD:	31.8
Millimolar Extinction Coefficient:	824.3	nmoles per OD:	1.2
Purity	Desalt	OD's	90.0
Tm (1 M Na+)	96	µg's*	2850
Tm (50 mM Na+)	77	nmoles	108
% GC	61	Coupling Eff.	98%
Notes:			

Primer 2:

Primer Name: UBI HSPB VER.2B
Researcher:
Primer Number: D0373B08 (B08)
Primer Length: 82
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG TCG AGA GGG GTC CAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	26872.4	µg per OD:	29.7
Millimolar Extinction Coefficient:	902.2	nmoles per OD:	1.1
Purity	Desalt	OD's	77.0
Tm (1 M Na+)	99	µg's*	2294
Tm (50 mM Na+)	78	nmoles	86
% GC	63	Coupling Eff.	98%
Notes:			

Primer 3:

Primer Name: UBI HSPA VER.3A
Researcher:
Primer Number: D0373B09 (B09)
Primer Length: 61
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTC GAG AGT TCC GCT CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	25160.2	µg per OD:	31.5
Millimolar Extinction Coefficient:	830.6	nmoles per OD:	1.2
Purity	Desalt	OD's	88.7
Tm (1 M Na+)	98	µg's*	2793
Tm (50 mM Na+)	76	nmoles	106
% GC	60	Coupling Eff.	98%
Notes:			

*-See Note about Quantities in Supporting Information.

[redacted]

LIFE TECHNOLOGIES

GIBCO BRL Custom Primers
Certificate of Analysis

Primer 4:

Primer Name: UBI HSPB VER.3B
Researcher:
Primer Number: D0373B10 (B10)
Primer Length: 82
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG AGC GGA ACT CTC GAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	26516.4	µg per OD:	29.7
Millimolar Extinction Coefficient:	901.3	nmoles per OD:	1.1
Purity	Desalt	OD's	83.2
Tm (1 M Na+)	99	µg's*	2478
Tm (50 mM Na+)	77	nmoles	92
% GC	62	Coupling Eff.	98%
Notes:			

Primer 5:

Primer Name: UBI HSPA VER.4A
Researcher:
Primer Number: D0373B11 (B11)
Primer Length: 96
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CGC TCT CGA CTC GAG AGT TCC GCT CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	30988.2	µg per OD:	31.7
Millimolar Extinction Coefficient:	976.3	nmoles per OD:	1.0
Purity	Desalt	OD's	89.3
Tm (1 M Na+)	100	µg's*	2833
Tm (50 mM Na+)	78	nmoles	91
% GC	61	Coupling Eff.	98%
Notes:			

Primer 6:

Primer Name: UBI HSPB VER.4B
Researcher:
Primer Number: D0373B12 (B12)
Primer Length: 97
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG AGC GGA ACT CTC GAG TCG AGA CGG GTC GAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	31791.4	µg per OD:	29.6
Millimolar Extinction Coefficient:	1070.6	nmoles per OD:	0.9
Purity	Desalt	OD's	97.1
Tm (1 M Na+)	100	µg's*	2883
Tm (50 mM Na+)	79	nmoles	90
% GC	62	Coupling Eff.	98%
Notes:			

* -See Note about Quantities in Supporting Information.

[redacted]

LIFE TECHNOLOGIES

9-007

Do Nucleobond preps of 5596, 5597,
4216, 4217, 4218 and 4219

→ Digest 5596 and 5597 w/ EcoRI as a check
make sure smaller frag. is ~2kb.

Digest 4216, 4217, 4218, 4219 w/ BglII/SalI
to check that the 168 bp frag is generated.

Digest 4216 w/ BsgI/XcmI to use as
accepting vector for ubiquitin versions 1-4.
Gel isolate on 1% agarose

Digest 5596 w/ NheI/NotI to isolate insert
(~1.6 kb) L4:BAASS. NotI and Nhe
Gel isolate on agarose cut in different buffer
Cut w/ NotI 1st Ø extra
and Et-OH ppt.
Cut w/ NheI

Mini-preps on BAASS:M
#s 5648 - 5665
Digest w/ EcoRI/PstI. Cut 1-18 also
Run on 10% acrylamide

→ Anneal Ubiquitin versions 1, 2, 3, and 4
oligos together.
Heat to 95°C for 5 min then stick on ice
Check these on a 10% acrylamide gel

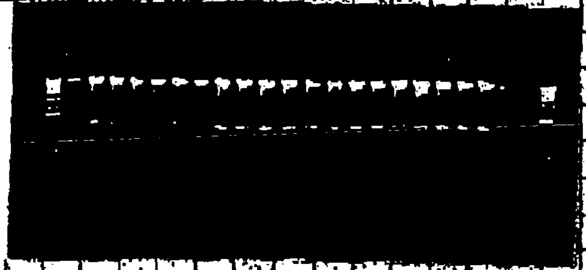
Run precut 3770 NheI/NotI on an agarose
gel to check it out [illegible signature]

[redacted]

13-004

Check Ubi variation w/ a H3/Whol digest (first 5 of each)

1 1Kb ladder
2-6 Ubi 1 7593-7597
7-11 Ubi 2 7561 - 7565
12-16 Ubi 3 7579 - 7583
17-21 Ubi 4 7597 - 7601
22 [redacted] NL/H3 7062 vector
23 6939 NL/H3 frag
24 1Kb ladder



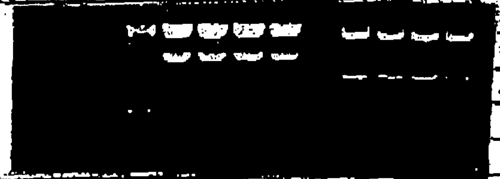
All look fine. Send these for sequencing

Sequencing data shows 7368 to be correct for GUS/IL2P

Make Avidin IL2P using 7368 construct

Digest 7424 (Avidin/3720) and 7368 (GUS/IL2P) with BamHI/XbaI

Gel: 1 1Kb ladder
2-5 7368 (GUS/IL2P) Bam/Xba
6 Skip
7-10 7424 (Avidin) Bam/Xba



Isolate large vector frag from GUS/IL2P and the smaller insert band from Avidin

Katherine Beifuss

[redacted]

Gus Assay

PURPOSE: TO QUANTITATE THE AMOUNT OF GUS IN CORN SEED

MATERIALS: READING PLATE - COSTAR EIA/RIA
READING PLATE - NUNC. FLUORONUNC POLYSORP
MU - 4 METHYLUMBELLIFERONE (SIGMA M-1508)
MUG - 4 METHYLUMBELLIFERONE B GLUCURONIDE (SIGMA M-9130)
MICROBALANCE
FLUORESCENCE MICROPLATE READER

PROCEDURE: USE PROTOCOL FOUND ON PAGE* 57 OF THIS NOTEBOOK (#58).

RESULTS: DATA FOUND BELOW. (BASED ON 20-MIN READINGS.

SAMPLE#	%TSP	SAMPLE#	%TSP
GSE 12020-4	.088	GSE 06030-1	0.087
-5	ND	-2	0.54
GSD 01120-1	ND	-3	0.61
-2	ND	-4	0.16
-3	ND	-5	0.06
-4	ND	" 0828-1	0.021
-5	ND	-2	0.002
GSE 15070-4	0.28	-3	0.027
" 050501	0.17	-4	ND
-2	0.015	-5	0.001
-3	0.010	" 07050-1	0.3
-4	0.174	-2	0.089
-5	0.010	-3	0.27
" 05090-1	0.043	-4	0.013
-2	0.014	-5	0.43
-3	0.001		
-4	0.004		
-5	0.004		
GSC 01010-1	0.005		
-2	0.010		
-3	0.009		
-4	0.60		
-5	~~ND~~ 0.48		

[redacted]

Investigator: Book # 58

Chris Brook Date:

Witness: Elizabeth Wilfong Date:

GUS ASSAY

SEE PURPOSE, MATERIALS, & PROCEDURE BELOW.

Purpose: To quantitate the amount of GUS in corn seed extracts.

Materials: [illegible]

Reagents: Lysis Buffer: [illegible]

Procedure: [illegible]

CB

[illegible]

CB

0 nM MU standard	[illegible] µl lysis buffer / well
1000 nM MU standard	12.5 µl of the 1:100 dilution + 87.5 µl lysis buffer / well
10,000 nM MU standard	12.5 µl of the 1:10 dilution + 87.5 µl lysis buffer / well
100,000 nM MU standard	12.5 µl of the 1 mM MU stock + 87.5 µl lysis buffer / well

CB

[redacted]

RESULTS: DATA FOUND BELOW. (10-MIN READINGS)

SAMPLE#	%TSP	SAMPLE#	%TSP	SAMPLE#	%TSP
GSG 01040-1	~~0~~ 0	GSG 01110-1	~~0.6~~ 0.06	GSC 01060-1	~~0~~ 0
-2	~~0.4~~ 0.04	-2	~~0.4~~ 0.04	-2	~~0~~ 0
-3	~~0.6~~ 0.06	-3	~~0~~ 0	-3	~~0~~ 0
-4	~~0.5~~ 0.05	-4	~~0.4~~ 0.04	-4	~~0~~ 0
-5	~~0.4~~ 0.04	-5	~~0.4~~ 0.04	-5	~~4.8~~ 0.5
GSD 02130-1	~~1.1~~ 0.1	GSC 01070-1	~~4.2~~ 0.4	GSC 01130-1	~~8.4~~ 0.8
-2	~~0.7~~ 0.07	-2	~~2.7~~ 0.3	-2	~~0.1~~ 0.01
-3	~~0.9~~ 0.1	-3	~~3.4~~ 0.3	-3	~~8.6~~ 0.9
-4	~~0~~ 0	-4	~~5.2~~ 0.5	-4	~~5.0~~ 0.5
-5	~~0.8~~ 0.1	-5	~~0.01~~ ~~0.008~~ 0.001	-5	~~0.7~~ 0.07
GSG 01020-1	~~0~~ 0	GSC 01040-1	~~0.1~~ 0.01	GSC 01110-1	~~0~~ 0
-2	~~0~~ 0	-2	~~5.1~~ 0.5	-2	~~9.2~~ 0.9
-3	~~0.12~~ 0.01	-3	~~0.3~~ 0.03	-3	~~0~~ 0
-4	~~0~~ 0	-4	~~0.3~~ 0.03	-4	~~0~~ 0
-5	~~0.2~~ 0.02	-5	~~0.04~~ 0.004	-5	~~4.6~~ 0.7
GSC 01030-1	~~0~~ 0				
-2	~~4.0~~ 0.4				
-3	~~4.2~~ 0.4				
-4	~~0.5~~ 0.05				
-5	~~4.5~~ 0.8				

Investigator: [signature] Date:

Book # 67

Witness: [signature] Date: